I hereby certify that this paper (along with any paper referred to as being attached or enclosed) is being transmitted by facsimile to the Patent and Trademark Office, facsimile no. (703) 305-8568, on the date shown below.

Dated: August 17, 2006 Signature: (Wendy A. Balabon)

Docket No.: 66221-0048
(PATENT)

**IN THE UNITED STATES PATENT AND TRADEMARK OFFICE**

In re Patent Application of:
Lawrence S. Young et al.

Application No.: 10/538,546 Confirmation No.: 5446

Filed: June 10, 2005 Art Unit: 1644

International Application No.: PCT/GB2003/005403 Examiner: Not Yet Assigned

Filed: December 10, 2003

For: CANCER IMMUNOTHERAPY USING POLYCOMB PROTEINS

**REQUEST FOR CORRECTED PATENT APPLICATION PUBLICATION**
**PURSUANT TO 37 CFR 1.221(b)**

RECEIVED
USPTO-PG PUBS
AUG 17 2006

Attention: Publishing Division
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Dear Sir:

Applicants hereby request correction of the publication of the application filed in this matter, as follows:

On June 10, 2005 Applicants filed a National Phase Application pursuant to 35 U.S.C. §371 derived from International Application no. PCT/GB2003/005403, filed December 10, 2003, along with a preliminary amendment. The U.S. Patent and Trademark Office published an application related to this matter on June 15, 2006.

In comparing the published application with the application as actually filed, Applicants noted that the last page of the Specification from the underlying international application was

omitted from the published application. The omission left out Examples 9 and 10 of the application as filed. Applicants hereby request that the omission be corrected. The errors were not in the application as filed by Applicants; accordingly Applicants believe that no fee is required.

Transmitted herewith is a copy of the last page of the specification as filed and a copy of the last page of the specification in the published application.

Applicants believe that no fee is due with this request. However, if a fee is due, please charge our Deposit Account No. 18-0013, under Order No. 66221-0048 from which the undersigned is authorized to draw.

Dated: August 17, 2006

Respectfully submitted,

By_____
James F. Kamp
Registration No.: 41,882
RADER, FISHMAN & GRAUER PLLC
Correspondence Customer Number: 10291
Attorney for Applicant

istration, mice are usually left at least 5 days prior to harvesting serum to measure humoral responses or the spleen or lymph nodes to measure cellular responses. Humoral responses can be detected using a number of standard procedures well known to those skilled in the art. These methods include immunoblotting, Western blotting, a BMI-1 specific ELISA and radioimmunoprecipatation. These procedures are well-known to those skilled in the art. The following Western blotting protocol can be used to detect anti-BMI-1 antibodies in murine sera. Mice immunised with BMI-1 were bled at various time points following the vaccination. For each sample, protein extracts from a cell line expressing high amounts of BMI-1 protein (MCF-7) are run on NuPAGE Bis-Tris pre-cast gel (Invitrogen), and transferred to a PVDF membrane using the Xcell II Blot module (Invitrogen) according to manufacturer instructions (30V constant for 1 h in NuPAGE transfer Buffer+10% methanol). After 1 h blocking with casein at RT, membranes are blocked to avoid non-specific binding of biotin/avidin. This blocking step is performed using the Vector Blocking kit by 10 min incubation at RT in Avidin D solution (2 drops of Avidin D from the kit in 10 ml TBS) followed by a brief wash and 10 minutes incubation at RT in biotin solution (2 drops of Biotin from the kit in 10 ml TBS). After blocking, membranes are stained using VECTASTAIN ABC-AmP kit (Vector) according to the manufacturer's instructions. Briefly, membranes are incubated for 30 min with various dilutions of mouse serum in PBS and washed. For all washings, 3 incubations of 4 min in casein solution (Vector) are performed, and all incubations are done at RT. Membranes are then incubated for 30 min with 10 ml of biotinylated anti-mouse IgG (Vector) diluted at 1.5 μg/ml in casein solution. After washing, membranes are incubated 10 min with VECTASTAIN ABC-AmP Reagent diluted 1:500 in casein solution, washed, equilibrated by 5 min incubation in 0.1M Tris buffer pH 9.5, and stained with the Chromogenic Substrate Development kit (Vector, cat No. AK-6401). For this staining, membranes are incubated in the staining solution (4 drops of each reagent from the kit in 10 ml of 0.1M Tris buffer pH 9.5) for 5 to 30 min and washed with water. To detect the anti-BMI-1 antibodies by radio-immunoprecipitation following can be used. Labeled recombinant BMI-1 can be produced by in vitro transcription and translation (Promega, UK) with $^{35}$S-methionine (Amersham, UK) from a plasmid encoding BMI-1 under the SP6 or T7 promoter. In vitro translated $^{35}$S-BMI-1 (20,000 cpm) is incubated overnight at 4° C. with 2 μl of diluted hyperimmune murine serum (1:25 to 1:500). Autoantibody-bound antigen is precipitated with 25 μl of 25% protein A-Sepharose with 25% protein G-Sepharose (Amersham-Pharmacia, UK) in Multiscreen-DP opaque 96-well filtration plates (Millipore, UK) and is washed 8 times with washing buffer (20 mM Tris-HCl, pH 7.4, containing 150 mM NaCl, 0.1% BSA, and 0.15% Tween-20) using a Millipore vacuum-operated 96-well plate washer (Millipore, UK). After washing, scintillation fluid is added directly to the 96-well plate and radioactivity counted on a TopCount 96-well plate beta counter (Packard).

[0076] Cellular immune responses can be demonstrated by demonstrating T cell-derived antigen specific cytolytic activity or IFNγ secretion in response to BMI-1 presenting target cells. Spleens are removed from mice 10 days after the last BMI-1 immunisation. The spleen cells are immediately cocultured with syngenic Bone Marrow-derived Dendritic Cells (BMDC) transfected with a BMI-1 encoding construct using a non-viral transfection system, or electroporation. BMDC transfected with an irrelevant cDNA are used as negative controls, as well as spleen cells from mice immunised with irrelevant cDNA and adenovirus. Coculture is performed in ELispot plates (Millipore) coated with IFN-γ mAb with $5\times10^5$ splenocytes/well and up to $1\times10^5$ BMDC/well. After 24 h co-culture in 200 μl of RPMI 10% FCS, Elispot plates are washed and stained according to manufacturer instructions and spots are counted using digital image analyser software. BMDC are generated by culturing mouse BM cells (flushed from femurs and tibia), depleted of T cells and erythrocytes (cocktail of anti-CD4, -CD8, -CD45R followed by lysis with guinea pig complement and red cell lysis buffer.), in 500 U/ml murine GMCSF and 1000 U/ml murine IL-4.

## EXAMPLE 7

### Anti-tumour Responses in Murine Model(s)

[0077] Mice are vaccinated against BMI-1 using protocols and procedures as described in Example 6. The vaccinated mice are then challenged with a BMI-1 expressing syngeneic tumour cell line. The anti-tumour activity of the anti-BMI-1 immunisation is demonstrated by observed protection from challenge with the BMI-1 expressing tumour. An appropriate tumour model for this study is the murine mammary cell line 4T1 in Balb/c mice.

## EXAMPLE 8

### Immunostaining of Primary Tumour Samples

Method

[0078] Embedded tissue sections were stained as follows: the sections were soaked in xylene for 5 mins, alcohol for 5 mins, returned to water, and then treated with 0.3% $H_2O_2$ in $H_2O$ for 15 mins followed by a wash in water. The sections were placed in EDTA buffer pH8 plus Tween20@ 65° C. on a hotplate stirrer (500 rpm) overnight (ALTER technique by G M Reynolds: Reynolds, G. M., Billingham, L. J., Gray, L. J., Flavell, J. R., Cocker, J., Scott, K., Young, L. S. and Murray, P. G. (2002) IL-6 expression in Hodgkin/Reed-Sternberg cells is associated with the presence of 'B' symptoms and failure to achieve complete remission in patients with advanced Hodgkin's disease. Br. J. Haematol. 118; 195-201). Slides were rinsed in water and were mounted onto a Sequenza™ (ThermoShandon, Runcorn, UK) and washed in TBS (Tris-buffered saline) pH 7.6. They were then incubated in primary anti-BMI-1 Ab (either a 1:10 dilution in TBS for 4 hrs for mC69 Ab (gift from Arie Otte, University of Amsterdam), or a 1:100 dilution in TBS for 1 hr for clone 229F6 mAb (Upstate Cell signalling Solutions, Milton Keynes, UK). Slides were then washed in TBS plus Tween20 and incubated in Dako EnVision™ secondary Ab (Dako UK Ltd, Ely, UK) for 30 mins. Slides were washed in TBS plus Tween20 and visualised by incubating in Vector NovaRED™ (Vector Laboratories, Peterborough, UK) for 10 mins. The slides were then counterstained in Mayers Haematoxylin for 10 secs, dehydrated, cleared and mounted.

Results

[0079] FIG. 5 shows the over-expression of BMI-1 in a number of tumour specimens. In all sections the nuclei of tumour cells can be seen to clearly stain positive for the presence of BMI-1. Positive staining tumour cells are indicated by an arrow(s) on each of the slides.

[ - - - - - - - - - - ] Insert Examples 9 and 10

**Example 9 CD8-mediated responses to EZH2 peptides in liver cancer patients**

***Method***

Putative cytotoxic T lymphocyte (CTL) epitopes derived from the EZH2 polycomb protein restricted by either HLA-A2, HLA-B27 or HLA-B44 were identified using the BIMAS algorithm.

These peptides were then used in standard ELISPOT assays for CTL activity in both patients

with liver cancer (HCC patients) and normal donors.

***Results***

The data shown in Figure 6 represents the results of such assays in peripheral blood lymphocyte samples depleted for CD4 cells. They demonstrate that robust CD8-restricted CTL responses to EZH2 are present at high levels in liver cancer patients and at reduced but significant levels in normal donors.

**Example 10 Peptide-specific CTL-dediated lysis of target cells**

***Method***

Two HLA-A2 restricted peptides from EZH2 (referred to as SQA and YMC) were used in a dendritic cell stimulation protocol to generate CTL clones. These clones were tested in chromium release cytotoxicity assays against the autologous lymphoblastoid cell line pulsed with the relevant peptide.

***Results***

The data in Figure 7 demonstrate that CTLs generated against both SQA and YMC EZH2 peptides are able to recognise and lyse target cells presenting the relevant peptide.